

Intravenous Delivery of a Bi-functional AAV Gene Therapy to Reduce Endogenous ApoE4 and Express ApoE2 in ApoE4 Humanized Mice

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INTRODUCTION

Apolipoprotein E (ApoE) genotype represents the most significant genetic risk factor for Alzheimer's disease (AD), with distinct allelic variants exerting differential effects on disease susceptibility and progression. The ApoE2 allele is associated with a reduced risk and offers a protective effect, whereas the ApoE4 allele markedly elevates the risk of developing AD and is linked to an earlier onset of symptoms. Consequently, therapeutic strategies aimed at modulating ApoE expression, such as adeno-associated virus (AAV)-mediated delivery of the protective ApoE2 isoform and/or knockdown of ApoE4 expression, are emerging as promising avenues for intervention in AD pathogenesis. Here, we describe the identification of siRNAs targeting APOE *in silico*, confirmation of potent siRNAs *in vitro*, and subsequent embedding of top performing siRNA sequences into proprietary amiRNA cassettes for *in vitro* and *in vivo* evaluation. We then demonstrate IV delivery of investigational AAV therapeutic candidates that combine expression of human APOE-targeting artificial microRNAs with concomitant ApoE2 expression via single dose IV-delivery using Voyager's proprietary, VCAP-Gen2 BBB-penetrant capsid. We observed significant reduction of endogenous ApoE4 in key AD-relevant brain regions of ApoE4 knock in mice, while significantly increasing expression of the ApoE2 isoform. We conclude that utilization of a novel BBB-penetrant capsid delivering a bi-functional payload effectively modulates the level of ApoE2 and 4, and is a viable approach that will be further evaluated in pre-clinical studies.

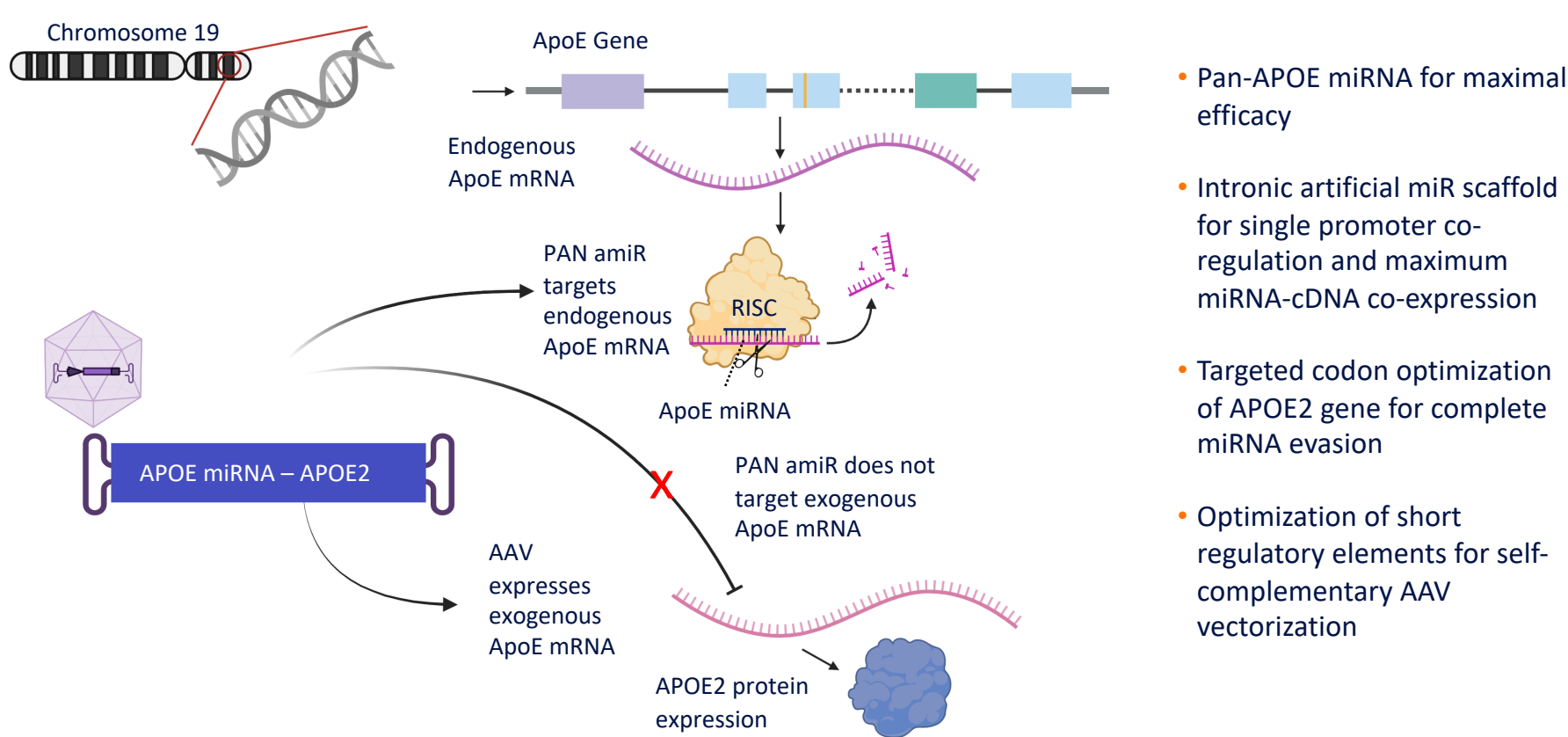
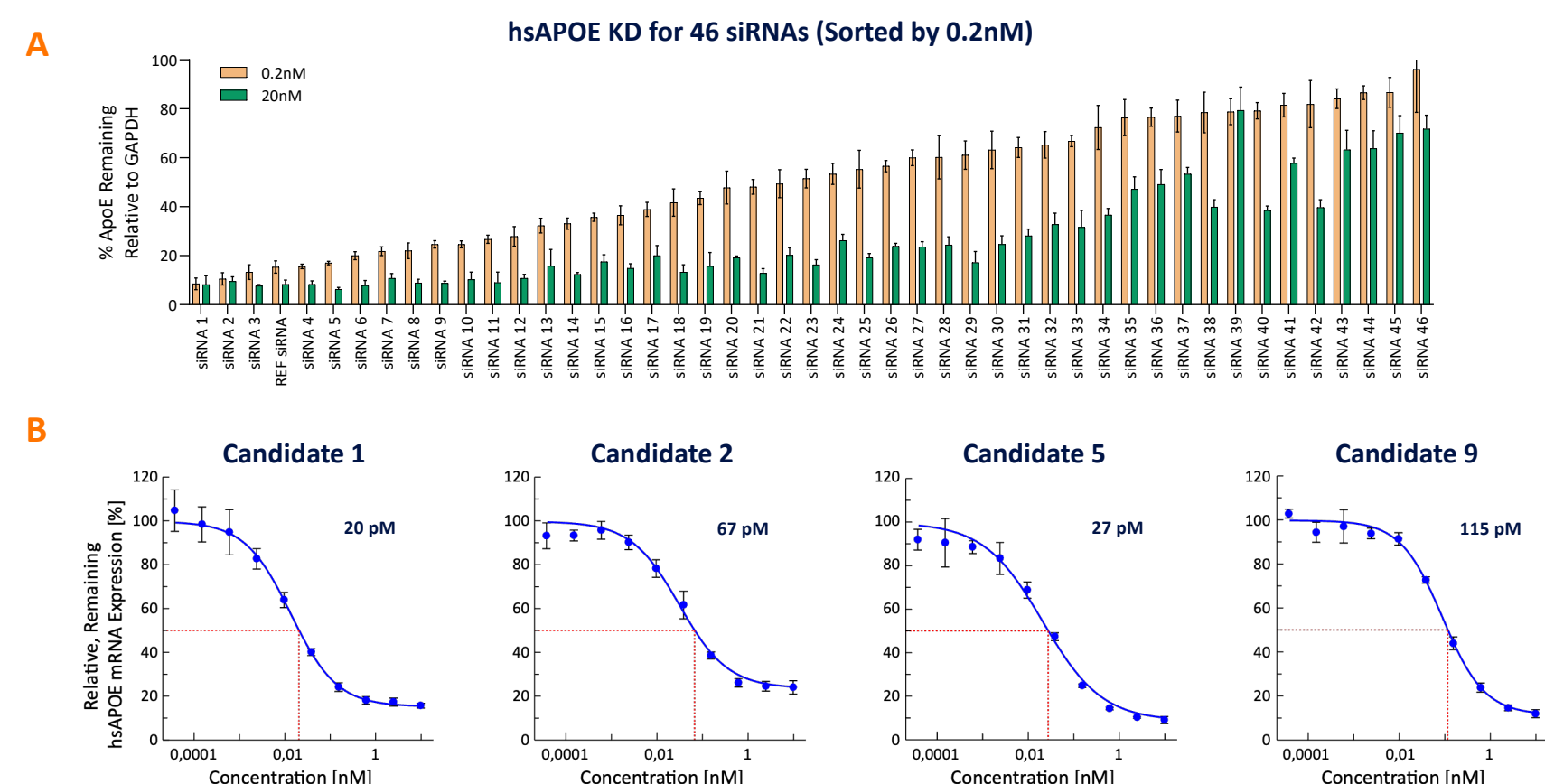
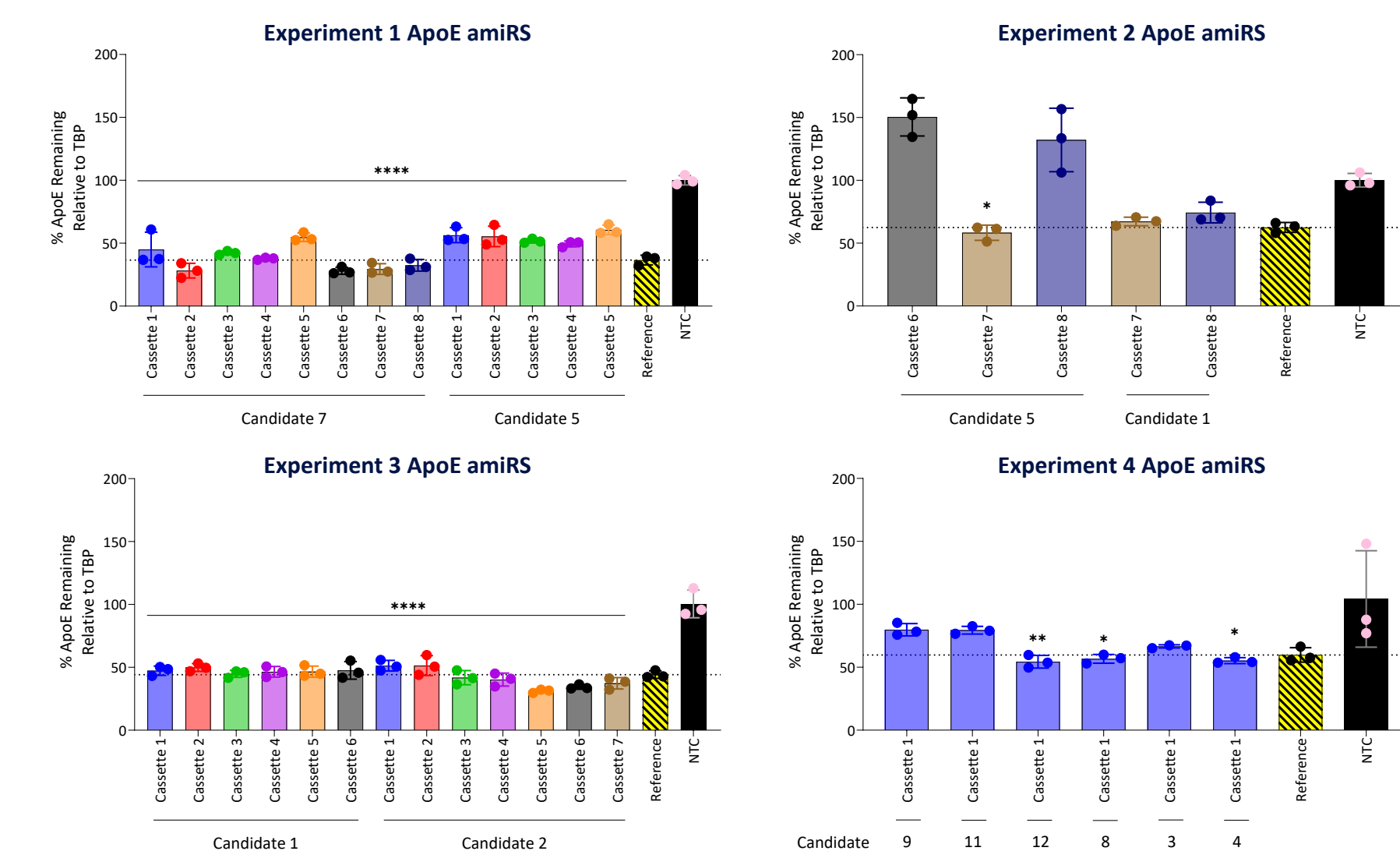


Figure 1. *In vitro* siRNA Screening Identifies Multiple Potent APOE-targeting siRNAs



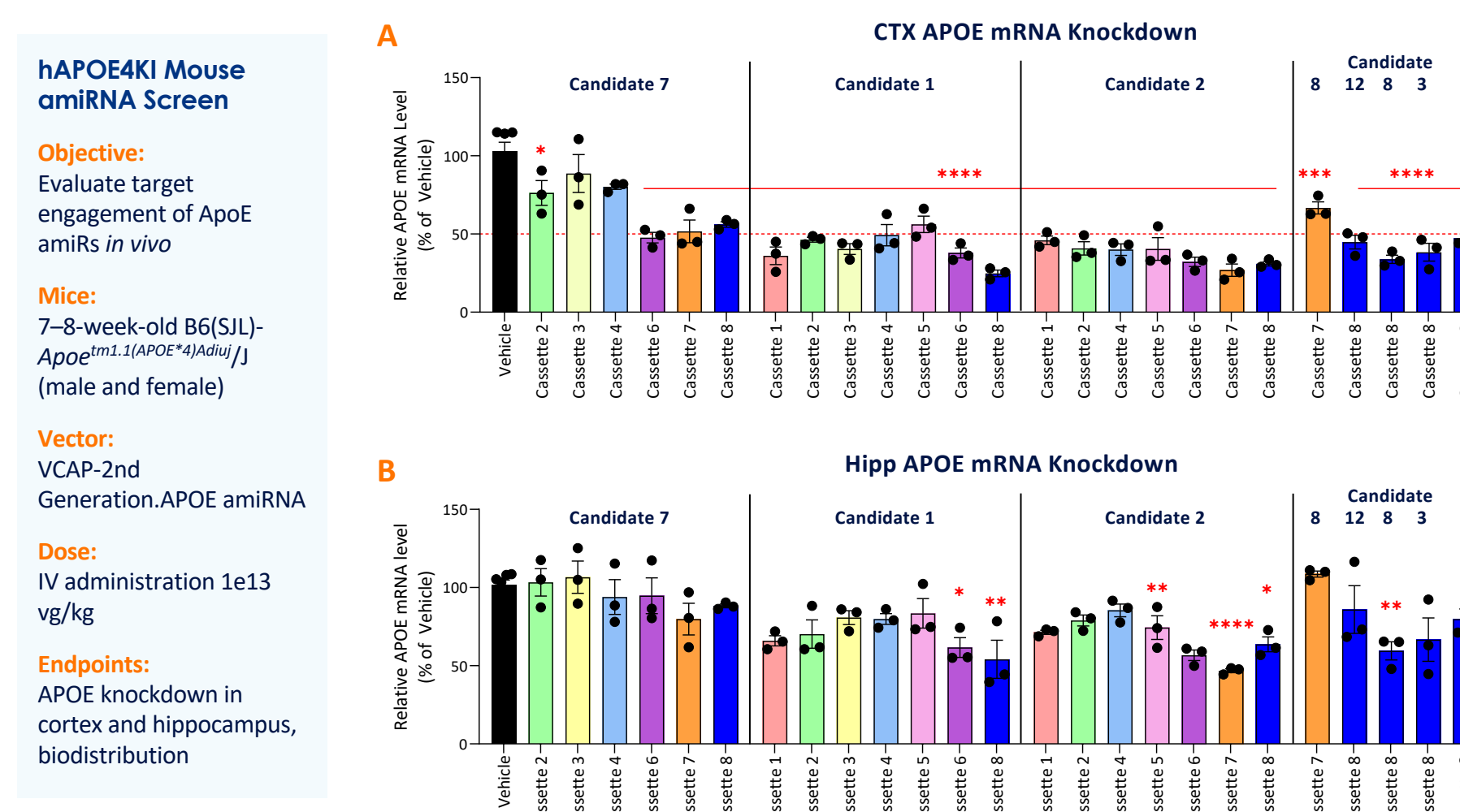
(A) ApoE mRNA silencing by potent siRNAs *in vitro*. 46 sequences selectively targeting APOE were designed, synthesized and evaluated in HepG2 cells. siRNAs were evaluated at two different concentrations, 0.2 nM and 20 nM. Following transfection, cells were harvested, and ApoE and GAPDH mRNA were quantified by RT-qPCR. (B) Dose response curves of the top performing siRNA candidates in HepG2 cells. 24 hr after transfection ApoE siRNA, cells were harvested, and ApoE and GAPDH mRNA were quantified by RT-qPCR.

Figure 2. Projected Therapeutic Level of hAPOE Silencing Achieved with amiRNAs *in vitro*



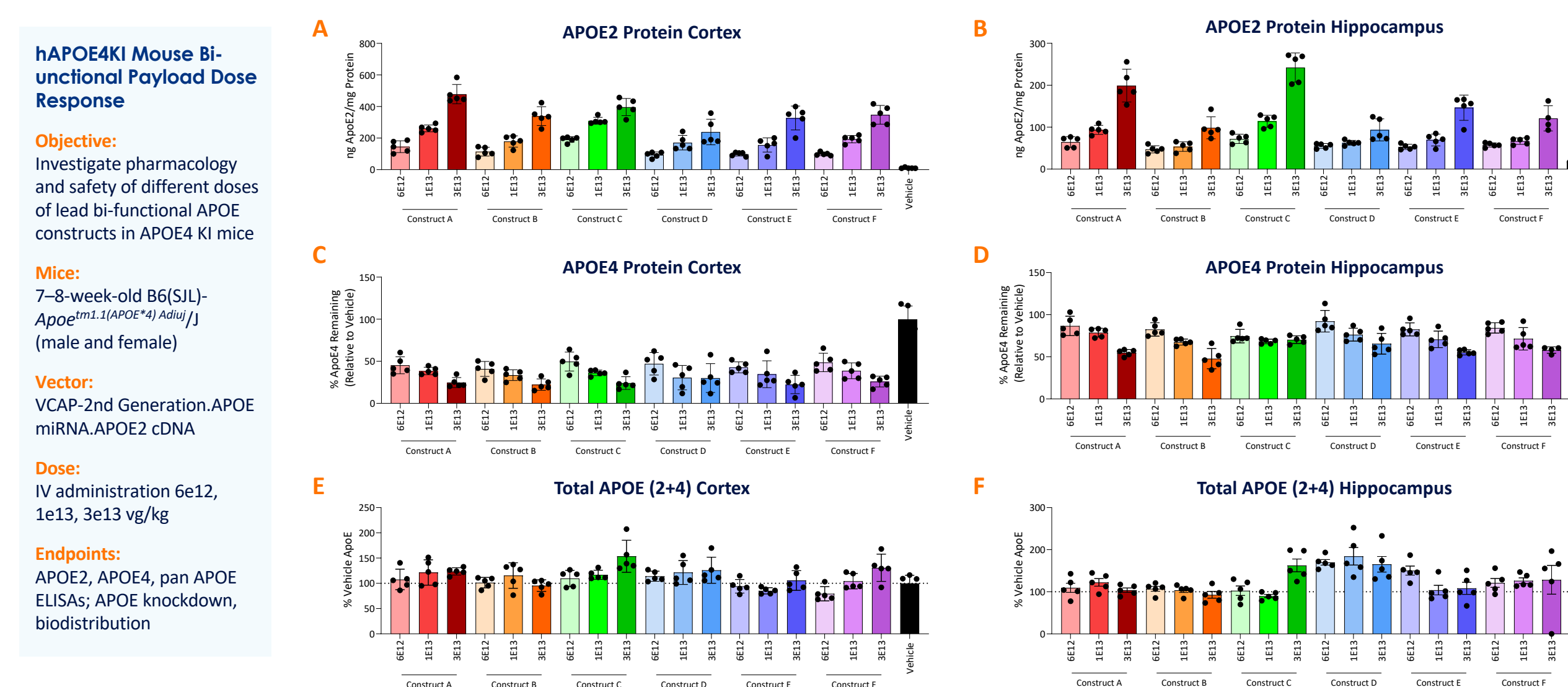
Best performing siRNA candidates were embedded in Voyager's proprietary amiRNA cassettes and evaluated in HEK293T cells in multiple experiments. 48 hr after transfection of 0.5 µg of APOE amiR expressing plasmids, cells were harvested, and APOE and TBP mRNA were quantified by RT-qPCR. A reference amiR was included as a positive control for each set tested. Several candidates exhibited knockdown as good, or better, than the knockdown achieved by the reference amiR. The best performing amiRs were selected for evaluation *in vivo*. *p<0.05, **0.01, ***0.001, ****0.0001 1-way ANOVA with Tukey's Multiple Comparisons.

Figure 3. Single IV-dose Delivery of APOE miRNA via VCAP Gen. 2 Capsids Results in Robust APOE mRNA Reduction in Key AD Brain Regions



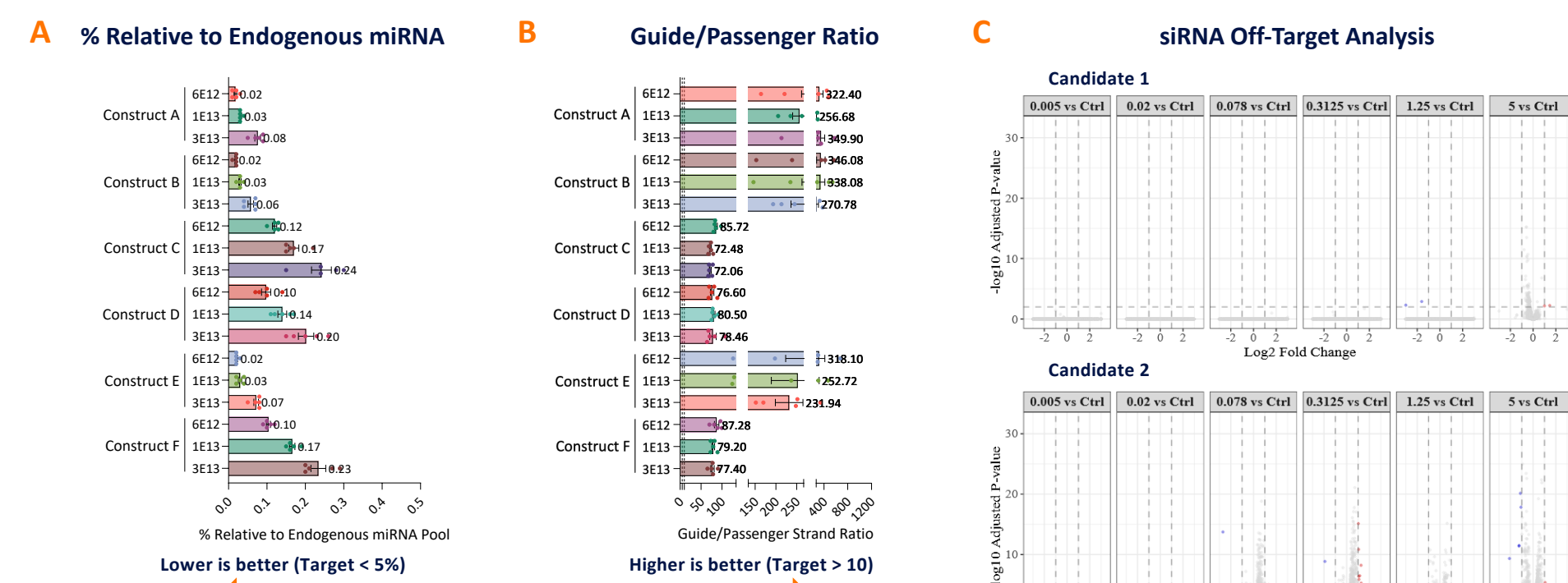
Substantial knockdown of hAPOE observed in cortex (A) and hippocampus (B) of APOE4KI mice 28 days after intravenous administration of AAV. hAPOE expression measured using multiplexed RT-qPCR with hAPOE expression level normalized to a host reference gene transcript with vehicle control group as the comparator. *p<0.05, **0.01, ***0.001, ****0.0001 1-way ANOVA with Tukey's Multiple Comparisons.

Figure 4. A Single IV Injection of Bi-functional Vector Leads to a Dose Dependent hAPOE4 Protein Reduction and hAPOE2 Delivery in Mouse Cortex and Hippocampus



Delivery of APOE2 and reduction of APOE4 in APOE4KI mice resulting in physiologically normal APOE levels with single IV administered bifunctional vector. Dose respondent APOE2 delivery in cortex (A) and hippocampus (B) measured with APOE2 specific ELISA. Recombinant APOE2 was used as a standard to quantify APOE2. Dose respondent APOE4 reduction in cortex (C) and hippocampus (D) measured with APOE4 specific ELISA, with the vehicle control group serving as a comparator. Total hAPOE (2+4) in cortex (E) and hippocampus (F) measured with pan hAPOE ELISA, with the vehicle control group serving as a comparator. Total APOE levels in treated groups are consistent with levels observed in vehicle group, indicating near physiological levels.

Figure 5. RNA sequencing Identifies Several Candidates with Favorable Safety Profile



CONCLUSIONS

- Several siRNA candidates demonstrate robust potency and selectivity for APOE *in vitro*
- Global miRNA profiling identifies several candidates with favorable amiR safety profile
- Vectorized amiRNA candidates produced significant APOE reduction in the cortex and hippocampus of APOE4KI mice
- Off-target analysis in HEK293T cells suggests selective APOE targeting at therapeutic miRNA concentrations, based on APOE *in vitro* IC₅₀
- IV injection of bifunctional APOE amiRNA-codon optimized APOE2 vectors produced dose dependent APOE4 protein reduction and APOE2 protein expression in the cortex and hippocampus of APOE4KI mice. Total APOE levels were modulated to physiological levels
- These data support the continued development of IV delivered AAV therapeutics that combine expression of human APOE-targeting artificial microRNAs with concomitant APOE2 expression